Citation:

Miller ER 3rd, Juraschek S, Pastor-Barriuso R, Bazzano LA, Appel LJ, Guallar E. Meta-analysis of folic acid supplementation trials on risk of cardiovascular disease and risk interaction with baseline homocysteine levels. *Am J Cardiol* 2010. Abstracted prior to publication.

Study Design:

Meta-analysis or Systematic Review

Class:

M - Click here for explanation of classification scheme.

Research Design and Implementation Rating:

POSITIVE: See Research Design and Implementation Criteria Checklist below.

Research Purpose:

The purpose of the meta-analysis was to synthesize findings from randomized controlled trials (RCTs) of folic acid supplementation to determine:

- Changes in homocysteine concentration
- Associations with cardiovascular disease (CVD) events
- Whether outcomes varied by baseline homocysteine concentration.

Inclusion Criteria:

- Studies indexed in MEDLINE from January 1966 to July 2009 or identified through manual searches for unpublished results of ongoing trials or conference presentations
- Randomized controlled trial design
- Folic acid supplementation
- Intervention at least six months
- Event numbers for control and intervention groups reported on: Cardiovascular disease, coronary heart disease, stroke, deep vein thrombosis, pulmonary emboli and all-cause mortality.

Exclusion Criteria:

Trials of pregnant women, children or patients with end-stage renal disease.

Description of Study Protocol:

Search Procedures

Randomized controlled trials published between January 1966 and July 2009 were identified through a MEDLINE search.

Study Quality

Study quality was not assessed, but the meta-analysis was limited to RCTs.

Interventions/Outcomes Abstracted

- Number of events of CVD, coronary heart disease (CHD), stroke, deep vein thrombosis, pulmonary emboli, and all-cause mortality during the longest follow-up period for folic acid-supplemented and control groups
    - Number of patients with myocardial infarction was abstracted from reports that did not describe the total number of cardiovascular events
- Results were recorded within each stratum from trials that stratified results by baseline homocysteine levels
    - Stratum-specific mean baseline homocysteine levels were abstracted or estimated from truncated sections of the overall within-trial homocysteine distribution
    - If number of events were not reported, stratum-specific relative risks (e.g., risk, rate or hazard ratios) for the primary clinical endpoint comparing supplementation vs. control were used.

Populations Included

All adult populations were included, except for pregnant women and patients with end-stage renal disease.

Data Collection Summary:

Information Abstracted

Folic acid supplementation, baseline homocysteine levels, and CVD events were abstracted. Three authors abstracted studies independently and discrepancies were resolved via consensus.

Analytic Methods

- Analyses were based on intention-to-treat
- Results from factorial trials were based on comparing all controls to all those who received folic acid supplementation, regardless of other factorial interventions
- Change in homocysteine was computed as change from baseline to end of intervention in the intervention group minus the change in the control group
    - Variance of net change was calculated assuming a common pooled correlation coefficient for all trials
- Risk ratios comparing controls to those receiving supplementation for primary clinical end-point, CVD, CHD, stroke and all-cause mortality were computed, along with their variances on the log scale
- Sensitivity analyses evaluated the influence of each trial on the pooled effects by removing each trial sequentially
- Potential confounding of country-specific folic acid food fortification was also examined.

Combining Findings

- Meta-analyses used random effects models
- Pooled estimates and 95% confidence intervals of net change in homocysteine and log-transformed risk ratio for each clinical outcome were calculated using inverse-variance weighted random-effects models

- The I^2 statistic was calculated for between-study heterogeneity
- The extended Egger test allowing for residual heterogeneity was used to assess publication bias and other small-study effects.

Baseline homocysteine interaction assessment

- For results stratified by baseline homocysteine, stratum-specific counts or relative risks (RR) were used
- Inverse-variance weighted random-effects meta-regression of log-transformed stratum-specific RRs on corresponding mean baseline homocysteine levels was used to pool results
    - Model slope quantified the folic acid-baseline homocysteine interaction as percent change in relative risk for the outcome for treatment vs. control per 1μmol/L increase in baseline homocysteine
- Pooled relative risks of supplementation vs. control were calculated for strata above and below the overall mean baseline homocysteine level of 12μmol/L.

Description of Actual Data Sample:

Number of Studies Identified

- A previous meta-analysis of folic acid supplementation trials included 12 studies from January 1966-July 2006
    - Four were excluded because they were of patients with end-stage renal disease
- The MEDLINE search of July 2006-June 2009 yielded 407 references
    - Of those, 397 were excluded because they were not an RCT, did not have CVD outcomes or folic acid supplementation, the follow-up was too short, or they were of patients who were pregnant, children or had end-stage renal disease
    - Of the 10 that were more closely examined, five were excluded because they either just detailed study design or were secondary publications
- The manual search yielded the results of one trial that have not yet been published.

Number of Studies Included

14

Sample Sizes and Participant Characteristics

- Nearly all studies were conducted in Western Europe or the US
- 12 of 14 trials were placebo-controlled and double-blind
- Except for one trial of women, males comprised 51.6-83.0% of the samples
- Total N =38,941 (range: 240-12,064)
- Mean age range: 52.2-68.9 years
- Average follow-up period: Six months to 7.3 years
- Nine trials recruited patients after CVD events (acute myocardial infarction, unstable angina, coronary artery stenting, angioplasty, non-disabling ischemic stroke, deep vein thrombosis, or pulmonary emboli); four enrolled those with pre-existing CVD or at high risk for it; one recruited those at low risk
- Folic acid supplementation range: 0.5-5mg per day
    - Three used folic acid alone, 11 supplemented in combination with vitamins B_{12} and/or B_6

- 13 of the trials used composite CVD outcomes as the primary clinical end-point (e.g., revascularization, nonfatal myocardial infarction, nonfatal stroke, sudden cardiac death, cardiovascular mortality, and all-cause mortality); one trial examined deep vein thrombosis and pulmonary emboli.

Summary of Results:

- Folic acid supplementation significantly lowered homocysteine levels, with a pooled net decrease of 2.9µmol/L (P<0.01; see table). There was strong heterogeneity across trials (I^2=91%)
- Supplementation had no effect on primary clinical end-points, with a pooled risk ratio of 1.02 (P=0.66; see table). The risk ratio was not altered dramatically by exclusion of each trial serially. There was moderate heterogeneity across trials (I^2=38%).
  - There was no evidence of publication-related bias (P=0.63)
  - There were no significant (NS) differences between countries with and without food fortification in baseline homocysteine, net homocysteine decrease, or primary clinical effects
- Supplementation had no effect on pooled risk ratios, and there was no evidence of heterogeneity or publication bias, for the following specific outcomes (see table):
  - Cardiovascular disease (P=0.42; I^2=0%)
  - Coronary heart disease (P=0.42; I^2=31%)
  - Stroke (P=0.43; I^2=25%)
  - All-cause mortality (P=0.78; I^2=0%)

Trial	Homocysteine Change (µmol/L; 95% CI)	Risk Ratios (95%CI) for Outcomes				
		Primary Clinical End-point	Cardiovascular Disease	Coronary Heart Disease	Stroke	All-cause Mortality
Baker et al, 2002	-1.5 (-2.3,-0.7)	1.91 (0.96,3.82)	-	1.91 (0.96, 3.82)	-	-
Schnyder et al, 2002	-2.9 (-3.6, -2.2)	0.68 (0.48, 0.96)	-	0.68 (0.48, 0.96)	-	0.52 (0.16, 1.70)
Lange et al, 2004	-3.6 (-4.3, -2.9)	1.53 (1.03, 2.28)	-	1.53 (1.03, 2.28)	-	1.01 (0.06, 16.1)
Liem et al, 2004	-	0.98 (0.69, 1.38)	-	0.98 (0.69, 1.38)	-	0.88 (0.30, 2.54)
Toole et al, 2004	-2.3 (-2.6, -2.0)	0.97 (0.84, 1.12)	0.98 (0.84, 1.16)	0.94 (0.73, 1.20)	1.04 (0.84, 1.29	0.86 (0.66, 1.11)

Liem et al, 2005	-2.6 (-3.2, -2.0)	0.85 (0.60, 1.21)	0.85 (0.60, 1.21)	1.25 (0.69, 2.26)	0.65 (0.27, 1.57)	0.68 (0.38, 1.21)
Bonaa et al, 2006	-3.5 (-3.9, -3.2)	1.07 (0.93, 1.22)	1.07 (0.93, 1.22)	1.05 (0.91, 1.21)	1.00 (0.68, 1.48)	1.02 (0.84, 1.24)
Lonn et al, 2006	-3.2 (-3.8, -2.6)	0.95 (0.85, 1.06)	0.95 (0.85, 1.06)	0.98 (0.85, 1.13)	0.76 (0.59, 0.96)	0.99 (0.88, 1.11)
den Heijer et al, 2007	-4.5 (-5.3, -3.8)	0.85 (0.58, 1.24)	-	-	-	-
Albert et al, 2008	-1.6 (-2.7, -0.5)	1.04 (0.92, 1.18)	1.04 (0.92, 1.18)	1.01 (0.86, 1.18)	1.14 (0.83, 1.57)	0.98 (0.83, 1.15)
Collins et al, 2008	-3.7 (-3.9, -3.5)	1.03 (0.97, 1.09)	1.03 (0.97, 1.09)	1.04 (0.97, 1.11)	1.01 (0.86, 1.20)	1.03 (0.95, 1.12)
Ebbing et al, 2008	-2.6 (-3.0, -2.3)	1.09 (0.91, 1.30)	1.09 (0.91, 1.30)	1.20 (0.95, 1.53)	0.72 (0.45, 1.17)	1.27 (0.90, 1.78)
Hodis et al, 2009	-2.1 (-2.7, -1.5)	0.81 (0.34, 1.93)	0.81 (0.34, 1.93)	-	-	0.20 (0.01, 4.11)
Imasa et al, 2009	-	1.40 (0.98, 2.01)	-	1.23 (0.61, 2.48)	-	1.18 (0.68, 2.04)
Pooled	*-2.9 (-3.4, -2.4)*	*1.02 (0.93, 1.13)*	*1.04 (0.94, 1.16)*	*1.04 (0.94, 1.16)*		*1.01 (0.95, 1.07)*

Studies that stratified by baseline homocysteine found that supplementation decreased CVD risk for participants with low baseline homocysteine but increased risk for those with high baseline levels

- For primary clinical end-point, pooled RR for supplementation increased 3.9% (95% CI: -3.0, 11.3%; P=0.27) for each 5µmol/L increase in homocysteine
- No heterogeneity of stratum-specific effects with respect to pooled linear trend was found (I^2=8%)
- When each trial was serially excluded, pooled RRs increased from 2.2% to 10.2%
- When comparing strata below and above the mean baseline homocysteine concentration, pooled relative risks differed significantly for primary clinical end-point (P=0.03)
    - <12µmol/L: RR=0.94; 95% CI: 0.86, 1.03; P=0.17
    - >12µmol/L: RR=1.06; 95% CI: 1.00, 1.13; P=0.06.

Trial	Baseline Homocysteine (µmol/L)		Relative Risk (95%CI)	Primary Clinical End-point
	Stratum	Mean		
Toole et al, 2004	Tertile 1 (<11.3)	8.0	1.14 (0.77, 1.67)	Recurrent ischemic stroke
	Tertile 2 (11.3-15.5)	13.4	1.15 (0.78, 1.69)	
	Tertile 3 (>15.5)	18.8	0.96 (0.69, 1.32)	
Liem et al, 2005	Quartile 1-3 (<13.7)	9.6	0.74 (0.50, 1.11)	Composite of recurrent nonfatal acute coronary syndrome, nonfatal stroke, transient ischemic attack and death
	Quartile 4 (>13.7)	16.6	1.37 (0.65, 2.87)	
Bonaa et al, 2006	<13.0	8.9	0.97 (0.79, 1.20)	Composite of recurrent myocardial infarction, stroke and cardiac death
	>13.0	17.2	1.27 (1.02, 1.66)	
Lonn et al, 2006	Tertile 1 (<10.0)	6.4	0.92 (0.67, 1.27)	Composite of nonfatal myocardial infarction, nonfatal stroke and CVD death
	Tertile 2 (10.0-12.7)	11.4	0.88 (0.65, 1.20)	
	Tertile 3 (>12.7)	16.8	1.04 (0.82, 1.32)	
den Heijer et al, 2007	Quartiles 1-3 (<12.6)	9	0.61 (0.35, 1.87)	Recurrent deep vein thrombosis or pulmonary emobolism
	Quartile 4 (>12.6)	15.5	1.12 (0.67, 1.87)	

Collins et al, 2008	<11.0	7.8	0.96 (0.85, 1.09)	Composite of revascularization, recurrent nonfatal myocardial infarction, stroke and CHD death
	11.0-14.0	12.5	1.11 (1.00, 123)	
	>14.0	17.8	1.00 (0.91, 1.09)	

Author Conclusion:

- Results from the meta-analysis showed that folic acid supplementation lowered homocysteine but had no overall effect on CVD, mortality or stroke
  - Folic acid may affect disease progression via homocysteine-independent pathways, a hypothesis that is consistent with other research in animal and *in vitro* models as well as clinical studies
- Supplementation in patients with higher baseline homocysteine levels was associated with greater risk of CVD events and lower risk for those with lower baseline levels
- Folic acid supplementation therefore should be not be recommended for CVD or stroke prevention.

Reviewer Comments:

- *Author-identified limitations:*
  - *Interactions could not be explored more fully because of differences in how composite clinical end-points were defined, differences in trial eligibility criteria, and inconsistent/incomplete reporting by baseline homocysteine levels*
  - *Subgroup and potential interactions may have occurred by chance alone.*
- *Women were underrepresented in the trials, and it is unclear whether the effects of folic acid supplementation differ by sex*
- *The authors did not address study quality or validity. However, the meta-analysis was restricted to RCTs, most of which were double-blind and placebo-controlled, so rigor is presumably high*
- *In the analyses stratified by baseline homocysteine, the authors did not include equalities in the strata definitions. The table in this abstract duplicates the authors' presentation.*

Research Design and Implementation Criteria Checklist: Review Articles

Relevance Questions

1.	Will the answer if true, have a direct bearing on the health of patients?	Yes
2.	Is the outcome or topic something that patients/clients/population groups would care about?	Yes

3.	Is the problem addressed in the review one that is relevant to nutrition or dietetics practice?	Yes
4.	Will the information, if true, require a change in practice?	N/A

Validity Questions

1.	Was the question for the review clearly focused and appropriate?	Yes
2.	Was the search strategy used to locate relevant studies comprehensive? Were the databases searched and the search termsused described?	Yes
3.	Were explicit methods used to select studies to include in the review? Were inclusion/exclusion criteria specified and appropriate? Were selection methods unbiased?	Yes
4.	Was there an appraisal of the quality and validity of studies included in the review? Were appraisal methods specified, appropriate, and reproducible?	Yes
5.	Were specific treatments/interventions/exposures described? Were treatments similar enough to be combined?	Yes
6.	Was the outcome of interest clearly indicated? Were other potential harms and benefits considered?	Yes
7.	Were processes for data abstraction, synthesis, and analysis described? Were they applied consistently across studies and groups? Was there appropriate use of qualitative and/or quantitative synthesis? Was variation in findings among studies analyzed? Were heterogeneity issued considered? If data from studies were aggregated for meta-analysis, was the procedure described?	Yes
8.	Are the results clearly presented in narrative and/or quantitative terms? If summary statistics are used, are levels of significance and/or confidence intervals included?	Yes
9.	Are conclusions supported by results with biases and limitations taken into consideration? Are limitations of the review identified and discussed?	Yes
10.	Was bias due to the review’s funding or sponsorship unlikely?	Yes